Review article

Targeted therapy against cytokines and chemokines in cancer

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Abstract:

Cytokines are a diverse group of non-antibody proteins that act as mediators between cells.They mediate & regulate amplitude and duration of immune & inflammatory responses.They are important specifically in host responses to infection, immune responses, inflammation, trauma, sepsis, cancer, and reproduction. The interaction between cytokines & chemokines and their receptors forms a network at tumour site responsible for tumour progression and induction of anti tumour responses and tumour rejection. These affect multiple pathways of tumour progression including leucocyte recruitment, cellular scenescence, tumour cell proliferation & survival, angiogenesis, invasion and metastasis. These also have role in cancer cachexia, act as anti tumour agents and as prognostic markers. Cytokine therapy is given to manipulate the immune response in such a way as to generate the appropriate immune effector cells to eradicate various solid tumors.There have been clinical trials executed involving the administration of interferon-gamma, interferon-alpha, interleukin-2, tumor necrosis factor-alpha, and Interleukin-12. Cytokine-based anticancer agents and antagonists to chemokines possess high potential for the development of therapeutic agents to treat various types of malignant cancer. Important ones are Aldesleukin, Sunitinib, Bevacizumab, Maraviroc and many more. Future applications of cytokine therapy may involve a combination of cytokines in the treatment of advanced malignancies. In particular, the combination of IL-2 and IL-12 may prove to be the most effective regimen.

Introduction

Cytokines are low molecular weight soluble proteins produced in response to microbes or other antigens. These are small cell-signalling protein molecules secreted by cells of immune system involved extensively in intercellular communications. These mediate and regulate amplitude and duration of immune and inflammatory responses. Categories of cytokines are Interferon, Interleukins, Tumour necrosis factor, Transforming growth factor, Colony stimulating factor, Growth factors. They play main role in hematopoiesis (CSF), inflammatory reaction (IL-1,TNF), chemotaxis (IL-8, MIP-1), immunostimulation (IL-12,IFN-γ), suppression (IL-10), angiogenesis (VEGF) and embryogenesis (TGF-β).

Various cytokines & their functions:

Name	Source	Function
1.Interferons (IFN)	INFα by leucocytes INFß by fibroblasts INFγ by NKcells,Th1 cells,CD 8 T cells	IFN-α & β increase MHC-1 expression on viral infected cells helping their recognition by CD8 T-cells & increase cytotoxic action of NK cells IFN-γ activates macrophages and promotes production of Th1
2.Interleukins (IL)	Helper CD4+ T lymphocytes & also by monocytes, macrophages & endothelial cells.	Promote development & differentiation of T,B and haematopoietic cells
3.Tumour Necrosis Factor (TNF)	Macrophages,CD4 lymphocytes,NK cells, mast cells,	TNF-α implicated in tumour regression, septic shock & cachexia.Potent pyrogen, causes fever by direct action or by stimulation of IL-1 secretion TNF-ß cytotoxic for wide range of tumour cells in vivo & in vitro.

		Also includes FasL, CD30L and TNF-related apoptosis inducing ligand (TRAIL).
4.Transforming Growth Factor (TGF)	Macrophages, brain cells, keratinocytes	TGF-α induces epithelial development. TGF-β plays a role in tissue regeneration, cell differentiation & regulation of immune system.Upregulated in some human cancers.
5.Colony Stimulating Factor (CSF)	macrophages, T cells, mast cells, endothelial cells & fibroblasts	Activates intracellular signaling pathways.Causes cells to proliferate & differentiate into a specific kind of blood cell (usually leucocyte).GM-CSF functions as a WBC growth factor & stimulates stem cells to produce granulocytes and monocytes

6.Growth Factors (PDGF,EGF,FGF, VEGF)	Fibroblasts,endothelial cells,platelets, macro-Phages,keratinocytes	typically act as signalling molecules between cells.Includes Bone Morphogenetic Proteins(BMPs), Epidermal Growth Factor (EGF),Erythropoietin (EPO),Thrombopoietin (TPO) & Platelet Derived Growth Factor (PDGF).BMPs stimulate bone cell differentiation while FGF & VEGF stimulate angiogenesis.

Chemokines

Chemokines are a family of small (8-10 kDa) proteins that act as chemoattractants for specific type of leucocytes. These are classified into 4 major groups according to arrangement of conserved cysteine (C) residues in major proteins[1].C-X-C chemokines (α) have 1 amino acid

residue separating the first two conserved cysteine residues. These act primarily on neutrophils .Eg. IL-8.C-C chemokines (β) have the first two conserved cysteine residues adjacent. Eg. Monocyte chemoattractant protein (MCP), eotaxin, Macrophage inflammatory protein (MIP-1α) & RANTES (regulated & normal T cell expressed & secreted).C chemokines (γ) lack two(1^{st} and 3^{rd}) of the four conserved cysteines.Eg. Lymphotactin relatively specific for lymphocytes. CX_3C chemokines contain 3 amino acids between two cysteines.

Cytokines & chemokines in cancer :

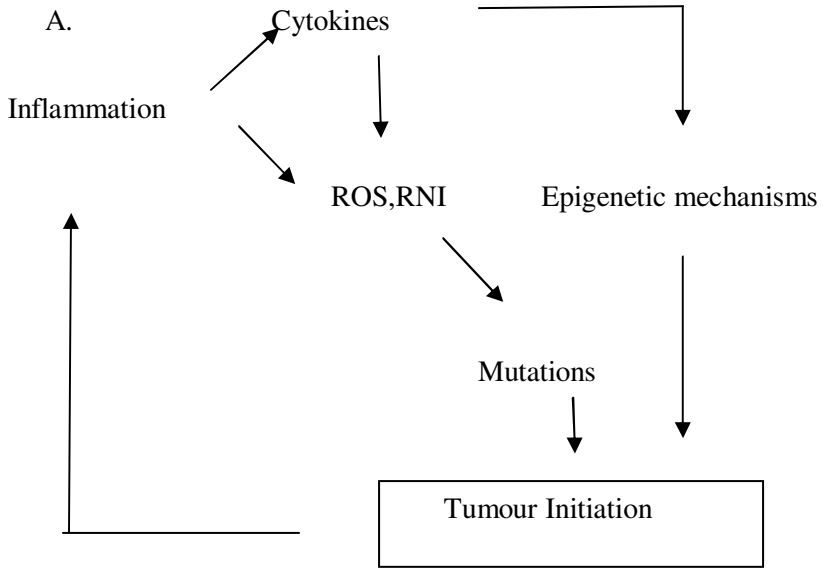
The tumour microenvironment consists of a variable combination of tumour cells,stromal

fibroblasts,endothelial cells and infiltrating leucocytes such as macrophages, T lymphocytes and dendritic cells.A variety of cytokines, chemokines and growth factors are produced in the local tumour environment by different cells.The interaction between cytokines, chemokines, growth factors and their receptors form a comprehensive network at tumour site which is responsible for tumour progression and induction of anti tumour responses and tumour rejection[2].

Cytokines that are produced in response to infection, inflammation and immunity can function to inhibit tumour development and progression. Also, cancer cells can respond to host derived cytokines that promote growth, attenuate apoptosis and facilitate invasion and metastasis.[3]

Role in tumour progression

Inflammation is the key component of tumour microenvironment and it has been proposed to represent 7th hallmark of cancer [4.] Chemokines are also part of the network of inflammatory mediators associated with neoplasia irrespective of pathogenesis[5].These affect multiple pathways of tumour progression including leucocyte recruitment, cellular scenescence, tumour cell proliferation & survival , angiogenesis and invasion and metastasis. Chemokines modulate tumour behaviour by three important mechanisms: regulation of tumour-associated angiogenesis, activation of a host tumour-specific immunological response, and direct stimulation of tumour cell proliferation in an autocrine fashion[6]. All of these mechanisms are promising points of cancer intervention.This suggests that chemokine antagonists could be important in development of new anti cancer therapies.



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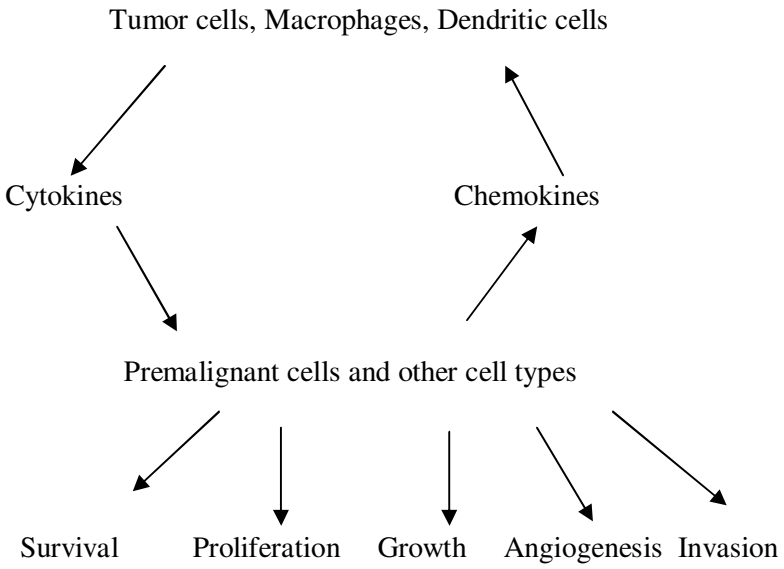

Role of inflammation in tumour initiation and promotion

A) Tumor initiation. Reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) produced by inflammatory cells may cause mutations in neighboring epithelial cells. Also, cytokines produced by inflammatory cells can elevate intracellular ROS and RNI in pre-malignant cells. In addition, inflammation can result in epigenetic changes that favor tumor initiation. Tumor-associated inflammation contributes to further ROS, RNI and cytokine production.

B) Tumor promotion. Cytokines produced by tumor infiltrating immune cells activate key transcription factors, such as NF-κB or STAT3, in pre-malignant cells to control numerous pro-tumorigenic processes, including survival, proliferation, growth, angiogenesis, and invasion. As parts of positive feed-forward loops, NF-κB and STAT3 induce production of chemokines that attract additional immune/inflammatory cells to sustain tumor-associated inflammation.[7]

Role in angiogenesis

The tumor-promoting functions of TNF-α may be mediated by its ability to induce proangiogenic functions, to promote the expression of matrix metalloproteinases (MMP) and endothelial adhesion molecules, and to cause DNA damage via reactive oxygen, the overall effect of which is promotion of tumor related processes.[8,9]

Tumour angiogenesis is crucial step in tumour development. Chemokines regulate angiogenesis directly through receptor expression on endothelial cells or indirectly recruiting leucocytes that provide angiogenic factors.CXCL5 and CXCL8 are potent promoters of angiogenesis. This correlates with progression

of breast, prostate, renal and lung carcinomas. The most frequently over expressed and best characterized chemokine receptor on tumour cells is CXCR4.It is associated with tumour progression and breast cancer metastasis[10].CCR7 is a potential marker to predict metastasis in breast and colorectal cancer.

Role in cancer cachexia

There is a role of pro-inflammatory cytokines, TNF, IL-1, IL-6, IFN-γ in the pathogenesis of cancer cachexia.Various efforts to block the development of cancer cachexia with anticyokine therapy will require inhibition of several pro-inflammatory cytokines simultaneously.[11]

Role as anti-tumour agents

Cytokines have been established as major mediators of anti tumour immunity .For example, IFN γ facilitates this anti tumour immunity by promoting antigen presenting cell (APC) mediated expansion of cytotoxic T cells and activated macrophages to release molecules like superoxide. IL-2 stimulates proliferation of primed cytotoxic T cells.IL-5 attracts eosinophils that produce cytotoxic proteins that disrupt cell membrane and induce cell death.IL-17 also plays a role in suppressing tumour growth and activity by promoting expression of MCP-1 and MIP-3α that recruit leucocytes and APCs to tumour to inhibit its growth.

Cytokines influence effectiveness of cancer treatments. Elevated cytokine levels have been associated with reducing anti-cancer activity of various treatments. Thus increasing proinflammatory cytokine levels can lead to NFKβ activation in cancer cells thus providing a mechanism for these cells to evade apoptosis.

Role as prognostic markers

Cytokine profile levels have been used to predict cancer prognosis as differential cytokine expression profiles have been correlated with disease progression.The switch from Th1 to Th2 cytokine expression has been associated with potential tumour metastasis and recurrence.IL-6 is often used as a prognostic marker for various cancers with abnormal elevated levels associated with poor prognosis.

Vicious cycle" of pro malignant activities

A proposed model for the potential role of the interactions between tumor cells and inflammatory elements in breast cancer progression. The expression of monocyte chemoattractants (CCL5 and CCL2) by breast tumor cells may induce monocyte infiltration to breast tumor sites. The resulting tumor-associated macrophages (TAM) may express promalignant mediators, such as tumor necrosis factor alpha (TNF-α). This inflammatory cytokine may further promote the expression of tumor-supporting factors by the tumor cells, including matrix metalloproteinases (MMP) and the monocyte chemoattractants CCL5 and CCL2. The elevated expression of these chemokines by the tumor cells may result in additional monocyte recruitment, and in the stimulation of TAM at the tumor site. TAM stimulation may give rise to promoted levels of expression of promalignant factors, such as MMP, angiogenic mediators and TNF-α. TAM-derived TNF-α may in turn further increase the expression of monocyte chemoattractants (e.g. CCL5, CCL2) by the tumor cells, and so on. This process may be aided by other functions of inflammatory cells/cytokines/chemokines (vascularization, release of growth factors,

etc.that eventually support the growth of the primary tumor and distant metastasis formation (possibly assisted by other chemokines, such as CXCL12)[13].

Role in metastasis

An important role for the CXCL12/CXCR4 axis in ovarian tumor metastasis was also identified and a correlation between the activity of this chemokine system and an enhanced intraperitoneal dissemination of epithelial ovarian cancer was described.[14]

Cytokine therapy for cancer

Cytokine therapy had been proven to be a novel therapeutic approach in treating patients with advanced malignancies. The purpose of this type of therapy is to manipulate the immune response in such a way as to generate the appropriate immune effector cells to eradicate solid tumors. This form of therapy is administrated only after the conventional form of therapies have been performed such as chemotherapy, radiotherapy, and surgery. But it can be given along with chemotherapy drugs(known as biochemotherapy) as in Stage IV melanoma. Various regimens of cytokine administration had been implemented in eradicating solid tumors in patients with melanoma and renal cell cancer. There have been clinical trials executed involving the administration of interferon-gamma, interferon-alpha, Interleukin-2, tumor necrosis factor-alpha, and Interleukin-12. Advances in cytokine therapy have been thwarted by the relatively high level of toxicity associated with the administration of cytokines. Common toxicities include nausea, vomiting, fever/chills, fatigue, and headache. Partial or complete tumor regression has been noted in some clinical trials which offer hope in treating advanced malignancies.

Interferons are used for treatment of viral diseases and cancer.Interferon-α is used in the treatment of hairy-cell leukaemia, AIDS-related Kaposi's sarcoma, follicular lymphoma, chronic myeloid leukaemia and malignant melanoma.Since the major pysiologic role of IL-2 is to promote the activation and proliferation of T and NK cells in an autocrine and paracrine manner[15] , it was delivered to patients with advanced cancer with the intention of eradicating the tumor for an extended period of time with only minimal toxicity to the patient. Modulation of immune responses by the use of recombinant cytokines or cytokine genes is one of the strategies for cancer therapy. Various commercial products of cytokines are available like Aldesleukin (recombinant human IL-2). It was first approved in 1992 for treatment of metastatic renal cell carcinoma. Interleukin-2 is used in the treatment of malignant melanoma and renal cell carcinoma. IL-7 in combination with human T cells exerts significant anticancer activity in human colon carcinoma in an *in vivo* study.[16]

GM-CSF is used to correct AIDS associated leukemia. Anti VEGF agents like Sunitinib (Sutent) are FDA approved for advanced renal cell carcinoma. Anti angiogenesis drugs like Bevacizumab (Avastin), Sorafenib (Nexavar), Cabozantinib (Cometriq) and Pazopanib (Votrient) are used in bladder cancer.

The most effective anti-inflammatory drugs had been those targeting TNF-α and IL-6. A humanized monoclonal anti-IL-6 antibody, ALD518 (Alder Biopharmaceuticals Inc, Bothell, WA, USA), may also benefit patients with

cancer-associated cachexia because its administration increases hemoglobin levels and prevents reduction in lean body mass in those with advanced non small cell lung carcinoma.Greater benefits may be conferred when TNF-α and IL-6 are targeted simultaneously. OHR Pharmaceutical Inc (New York, NY, USA) had developed the broad-spectrum peptide nucleic acid immune modulator drug, OHR/AVR118, which targets both TNF-α and IL-6 and maintains immune homeostasis. In a Phase II study, eight of 21 enrolled patients with advanced cancer completed the study, and showed an improvement in anorexia, dyspepsia, strength (assessed by grip strength), and depression.[17]

A humanized anti-IL-6 antibody (BMS-945429) had been shown to be safe and well tolerated during early clinical studies in patients with non small cell lung carcinoma, with treatment improving lung symptoms and reversing fatigue, with a trend towards a decrease in loss of lean body mass.[18] These findings are consistent with the results of a Phase II trial that assessed selumetinib (an inhibitor of MAPK1 and IL-6 secretion) in 20 patients with cholangiocarcinoma.[19] Overall, 84% of patients in this trial showed a mean muscle gain of 2.3 kg.[20]

Combination cytokine therapy offers promise in the treatment of advanced malignancies. A rational strategy might include low-dose continuous infusions of IL-2 to expand NK cells in vivo, followed by administration of IL-12 to augment NK cell cytolytic activity. TRAIL only induces cell death in malignant cells, making this a promising system for molecularly targeted therapies. There is growing evidence that this cytokine/receptor system is particularly active in high grade sarcomas such as Ewing's sarcoma[21] and rhabdomyosarcoma, but this has not yet translated into a clinical trial.

Anti- chemokines for cancer

Antagonists to chemokines receptors like CCR1, 2 ,CXCR 2, 3 etc are in therapeutic trials for management in asthma, chronic obstructive pulmonary disease,allergic diseases and transplant rejection.CCR5 antagonists like Maraviroc and Vicriviroc are useful adjuvant antimetastatic therapies for breast basal tumours. Antichemokine activity has been also identified in some natural compounds. Soyabean and cruciferous vegetables had been implicated in the protection against spontaneous and carcinogen-induced cancers although the mechanisms for this anticarcinogenicity are not fully elucidated.[22] Newer drugs still in the pipeline are MDX-1338 (Medarex) for acute myeloid leukemia and BKT140 (Biokine) for multiple myeloma.[23]

Newer strategies

A variety of innovative strategies for delivery of therapeutic cytokines have been promising in treatment of malignancy. These include cytokine-antibody fusion molecules (immunocyt-okines),recombinant viral vectors to deliver cytokine genes, transgenic expression of cytokines in whole tumor cells, and chemical conjugation to polyethylene glycol (PEGylation) to improve the kinetics of the Cytokine.

To summarize, cytokine-based anticancer agents possess high potential for the development of therapeutic agents to treat or manage various types of malignant cancer. [24] The clinical trials that have been conducted thus far have made a major impact in the treatment of cancer by

providing more information about the cytokine and chemokine network and how we can manipulate it in such a way as to combat malignancies.

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